REMARKS

It is respectfully requested that this application be reconsidered in view of the following remarks and that all of the claims remaining be allowed.

Claim amendments

Claims 1-20 have been canceled with prejudice or disclaimer. Applicants expressly reserve the right to file one or more continuing applications directed to the canceled subject matter.

New claims 21-32 have been added. Support for the new claims can be found, for example, as listed in Appendix A.

No new matter has been introduced by these amendments. The Examiner is requested to enter these amendments.

Restriction requirement

In the Office Action, the Examiner required the following restriction under 35 U.S.C. 121:

I. Claims 1-7, 9-10, drawn to polynucleotides, vectors, host cells and methods of making a polypeptide, classified in class 435, subclass 69.1, for example.

II. Claim 8, drawn to a method of forming a duplex, classified in class 435, subclass 504, for example.

III. Claims 11-12, 15, drawn to binding compounds, classified in class 530, subclass 387.1 for example.

IV. Claims 13-14, drawn to methods of using the binding compound, classified in class 436, subclass 501, for example.

V. Claims 16-18, drawn to a polypeptide, classified in class 530, subclass 350, for example.

VI. Claims 19-20, drawn to a method of modulating physiology of a cell, involving agonist of a primate IL-B50, classified in class 435, subclass 375, for example.

VII. Claims 19-20, drawn to a method of modulating physiology of a cell, involving antagonist of a primate IL-B50, classified in class 435, subclass 375, for example.

In response, Applicants elect Group V without traverse. Although all the original claims have been canceled, new claims 21-25 and 31-32 correspond to Group V.

Statement under 37 C.F.R. §1.607(c):

New claims 21-32 correspond substantially to claims 1, 3-8 and 10-14 of U.S. Patent No. 6,555,520 to Sims et al. They are being submitted to meet the requirements of 35 U.S.C. §135(b).

The remaining information required by 37 C.F.R. §1.607(c) will be submitted in due course. In the event that this application is reached for action by the Examiner prior to the submission of the remaining Rule 607 requirements, the Examiner is hereby requested to telephone the undersigned at 650-622-2300 (extension 2340).

Early examination of this application on the merits is earnestly solicited.

Respectfully submitted,

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Date: September 19, 2003

Appendix A
Exemplary Support for the New Claims

New claims	Exemplary support
21. An isolated polypeptide selected from the group consisting of: (a) a polypeptide encoded by a nucleic acid molecule having SEQ ID NO: 3; and	Sequence listing: SEQ ID NO:3 encodes SEQ ID NO:4; Page 12, lines 13-15: Purified IL-B50 Primate, e.g., human, IL-B50 amino acid sequence, is shown as one embodiment within SEQ ID NO: 2 or 4.
(b) a polypeptide encoded by a nucleic acid molecule which hybridizes to the complement of the polynucleotide having SEQ ID NO: 3 under conditions of about 100 mM salt and 60°C, wherein said polypeptide is capable of binding an IL-B50 receptor.	Page 33, line 31 to page 34, line 2: Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions; Page 31, lines 3-12: Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, usually in excess of about 37° C, typically in excess of about 55° C, 60° C, 65° C, or preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1000 or 600 mM usually less than about 400 mM, typically less than about 250 mM, preferably less than about 150 mM, including about 100, 50, or even 20 mM.

New claims	Exemplary support
22. A purified IL-B50 polypeptide wherein the polypeptide comprises SEQ ID NO: 4, or a fragment thereof,	Page 12, lines 25-28: As used herein, the term "human soluble IL-B50" shall encompass, when used in a protein context, a protein having amino acid sequence corresponding to a soluble polypeptide shown in SEQ ID NO: 2 or 4, or significant fragments thereof.
capable of binding IL-B50 receptors.	Page 18, lines 12-14 (emphasis added): In vitro assays of the present invention will often use *isolated protein, soluble fragments comprising receptor binding segments of these proteins*, or fragments attached to solid phase substrates. Page 18, lines 18-20 (emphasis added): This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the cytokine, or *receptor binding fragments* compete with a test compound. Page 40, lines 21-22: Helices A and D are most important in receptor interaction, with the D helix the more important region.

New claims	Exemplary support
23. A purified IL-B50 polypeptide comprising an amino acid sequence that is at least about 80% identical to the amino acid sequence of SEQ ID NO: 2, or a fragment thereof,	Page 12, lines 25-28: As used herein, the term "human soluble IL-B50" shall encompass, when used in a protein context, a protein having amino acid sequence corresponding to a soluble polypeptide shown in SEQ ID NO: 2 or 4, or significant fragments thereof. Page 15, line 29 to page 16, line 2: Identity measures will be at least about 35%, generally at least about 40%, often at least about 50%, typically at least about 60%, usually at least about 70%, preferably at least about 80%, and more preferably at least about 90%.
wherein the polypeptide is capable of binding IL-B50 receptors.	Page 18, lines 12-14 (emphasis added): In vitro assays of the present invention will often use *isolated protein, soluble fragments comprising receptor binding segments of these proteins*, or fragments attached to solid phase substrates. Page 18, lines 18-20 (emphasis added): This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the cytokine, or *receptor binding fragments* compete with a test compound. Page 40, lines 21-22: Helices A and D are most important in receptor interaction, with the D helix the more important region.

New claims	Exemplary support
24. A purified human IL-B50 polypeptide comprising an amino acid sequence that is at least 80% identical to amino acids 1 through 131 of SEQ ID NO: 4, or a fragment thereof, wherein the polypeptide is capable of binding an IL-B50 receptor.	Page 27, line 30 to page 28, line 1 (emphasis added): Said biologically active protein or polypeptide can be an intact antigen, or fragment, and have an amino acid sequence disclosed in, e.g., SEQ ID NO: 2 or 4, particularly *a mature, secreted polypeptide*. Page 10, lines 24-26: The signal sequence probably is about 33 residues, and would run from the met to about thr. See SEQ ID. NO: 1 and 2; supplementary sequence provides SEQ ID NO: 3 and 4.
25. A composition comprising the polypeptide of claim 22, 23, or 24, and a physiologically acceptable diluent or carrier.	Page 37, line 29 to page 38, line 1: IL-B50, antagonists, antibodies, etc., can be purified and then administered to a patient, veterinary or human. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents,...

New claims	Exemplary support
26. A method of stimulating lymphoid proliferation, comprising incubating lymphoid cells with the polypeptide of claim 22, 23, or 24.	Page 10, lines 13-16: It is likely that IL-B50 has either stimulatory or inhibitory effects on hematopoietic cells, including, e.g., lymphoid cells, such as T-cells, B-cells, natural killer (NK) cells, macrophages, dendritic cells, hematopoietic progenitors, etc. Page 37, lines 11-12: In particular, the cytokine should mediate, in various contexts, cytokine synthesis by the cells, proliferation, etc. Page 2, lines 10-11: Some of these factors are hematopoietic growth and/or differentiation factors, e.g., stem cell factor (SCF) and IL-7. Page 11, lines 1-3: The structural homology of IL-B50 to related cytokine proteins suggests related function of this molecule. IL-B50 is a short chain cytokine exhibiting sequence similarity to IL-7.
27. The method of claim 26, further comprising incubating the lymphoid cells with IL-7.	Page 9, lines 1-6: The invention embraces a method of modulating physiology or development of a cell or tissue culture cells comprising contacting the cell with an agonist or antagonist of a primate IL-B50. The method may be where: the contacting is in combination with an agonist or antagonist of IL-7; or the contacting is with an antagonist, including a binding composition comprising an antibody binding site which specifically binds an IL-B50.

New claims	Exemplary support
28. A method of stimulating lymphopoietic development comprising incubating progenitor cells with the polypeptide of claim 22, 23, or 24.	Page 55, lines 2-9: The effect on proliferation or differentiation of various cell types are evaluated with various concentrations of cytokine. A dose response analysis is performed, in certain cases in combination with the related cytokine IL-7 and/or stem cell factor. In particular, IL-7 exhibits strong effects on lymphopoietic development and differentiation. The IL-B50 will be tested on cord blood cells to see if it has effects on proliferation or differentiation of early progenitor cells derived therefrom. Page 10, lines 13-16: It is likely that IL-B50 has either stimulatory or inhibitory effects on hematopoietic cells, including, e.g., lymphoid cells, such as T-cells, B-cells, natural killer (NK) cells, macrophages, dendritic cells, hematopoietic progenitors, etc.
29. The method of claim 28, wherein the progenitor cells are bone marrow-derived stem cells.	Page 55, lines 9-11: Preferably, the cells are early precursor cells, e.g., stem cells, originating from, e.g., cord blood, bone marrow, thymus, spleen, or CD34+ progenitor cells.
30. The method of claim 29, further comprising incubating the bone marrow-derived stem cells with IL-7.	Page 55, lines 2-5: The effect on proliferation or differentiation of various cell types are evaluated with various concentrations of cytokine. A dose response analysis is performed, in certain cases in combination with the related cytokine IL-7 and/or stem cell factor.

New claims	Exemplary support
31. The polypeptide of claim 22 or 23, wherein the polypeptide is a fusion protein.	Page 19, lines 8-9: Fusion polypeptides between IL-B50s and other homologous or heterologous proteins are also provided.
32. The polypeptide of claim 31 wherein the fusion protein comprises an Fc domain.	Page 45, lines 28-31: For example, means to label the IL-B50 cytokine without interfering with the binding to its receptor can be determined. For example, an affinity label can be fused to either the amino- or carboxyl-terminus of the ligand. Such label may be a FLAG epitope tag, or, e.g., an Ig or Fc domain.

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Some drugs and medical devices presented in this publication have Food and Drug Administration (FDA) clearance for limited use in restricted research settings. It is the responsibility of the health care provider to ascertain the FDA status of each drug or device planned for use in their clinical practice.

cell surface receptors. Based on this operational type of definition, it is clear that the distinction between cytokines, growth factors, and hormones often may be imprecise. As a generalization, cytokines and growth factors can be thought of quite similarly, except that molecules involved in host defense that act on white blood cells (leukocytes) are generally called cytokines, whereas those that act on other somatic cell types are more typically described as growth factors. However, there is a major difference between cytokines and hormones. Cytokines generally act locally. For example, in the interaction between a T cell and an antigen-presenting cell, cytokines are produced and usually exert potent actions only locally, and have rather limited half-lives in the circulation. In contrast, after their release, hormones are generally disseminated by the bloodstream throughout the body, acting on a wide range of distal target organs.

In the immune system, terms such as "lymphokines" and "monokines" originally were used to identify the cellular source for the cytokine (1). Thus, interleukin-1 (IL-1), which was first recognized to be made by monocytes, was a monokine, whereas IL-2, which was first described as a T-cell growth factor, was a lymphokine. A major limitation of this nomenclature became evident when it was recognized that many of these lymphokines and monokines were in fact produced by a wide spectrum of cell types, resulting in the adoption of the term "cytokine," first coined by Stanley Cohen in 1974 (2,3). The term, in effect, refers to a factor made by a cell ("cyto") that acts on target cells. The range of actions of cytokines are diverse, including the abilities to induce growth, differentiation, cytolytic activity, apoptosis, and chemotaxis. The term "interleukin" refers to cytokines that are produced by one leukocyte and act on another leukocyte (4). In many cases, however, some interleukins (e.g., IL-1 and IL-6) are additionally produced by other cell types and can act on other cell types, and IL-7 is produced by stromal cells rather than by typical leukocytes.

Among the many different cytokines, the type I cytokines share a similar four α-helical structure, as detailed below, and correspondingly, their receptors also share characteristic features that have led to their description as the cytokine receptor superfamily, or type I cytokine receptors (5–8). Although many of the interleukins are type I cytokines, not all are. For example, of the proinflammatory cytokines IL-1, tumor necrosis factor (TNF)-α, and IL-6, IL-6 is a type I cytokine, whereas IL-1 and TNF-α are not (IL-1 and TNF-α are discussed in Chapter 22). One interleukin, IL-8, is a chemokine (see Chapter 22). Thus, the term "interleukin" refers to a relationship to leukocytes; in contrast, the characterization of a cytokine as a type I cytokine not only has implications regarding its three-dimensional structure, but also has implications related to the structure of its receptor and mechanisms of signal transduction.

In addition to molecules that primarily are of immunologic interest, other extremely important proteins, including growth hormone, prolactin, erythropoietin, thrombopoietin, and leptin, are also type I cytokines and their receptors are members of the same superfamily. As detailed below, these nonimmunologic cytokines share important signal transduction pathways with the type I cytokines of immunologic interest. Thus, the grouping in this chapter emphasizes evolution and signaling pathways, rather than common functions. Hence, although IL-6 exerts many overlapping actions with IL-1 and TNF-α, these latter molecules will be discussed elsewhere because the signaling pathways they use are very different from those common to type I cytokines and their receptors. This raises the important concept, however, that different end functions can be mediated via more than one type of signaling pathway.

The field of interferon (IFN) research has developed in parallel to the cytokine field. IFNs were first recognized as antiviral agents, and as such have been the source of great excitement both for basic science and for potential clinical uses. Over time, it has become clear that the type I cytokines and IFNs share a number of features that now for the first time result in their being addressed together in one chapter in this text. Correspondingly, it is noteworthy that the International Interferon Society changed its name to the International Society of Interferon and Cytokine Research and that the International Cytokine Society focuses on the IFNs, as well as cytokines, together emphasizing the importance of the common themes of IFNs and cytokines that will be the subject of part of this chapter.

TYPE I CYTOKINES AND RECEPTORS

Type I Cytokines: Structural Considerations

Despite the existence of extremely limited amino acid sequence similarities between different type I cytokines, it is striking that all type I cytokines whose three-dimensional structures have been solved (by nuclear magnetic resonance or x-ray crystallographic methods) have similar structures (5–8). Moreover, type I cytokines whose structures have not yet been solved also appear (based on modeling and comparison with the solved structures) to achieve similar three-dimensional structures (5–8). These cytokines are appropriately described as four α-helical bundle cytokines because their three-dimensional structures contain four α-helices (Fig. 1). The first two and last two of these α-helices are each connected by long-overhand loops. This results in an "up-up-down-down" topologic structure because the first two helices (A and B) can be oriented in an up orientation and the last two helices (C and D) can be oriented in a down orientation, as viewed from the NH_2- to COOH-terminal direction. As shown in Fig. 1, the N and C termini of the cytokines are positioned on the same part of the molecule.

Type I cytokines can be grouped as either short-chain or long-chain four α-helical bundle cytokines, based on their size (8). The

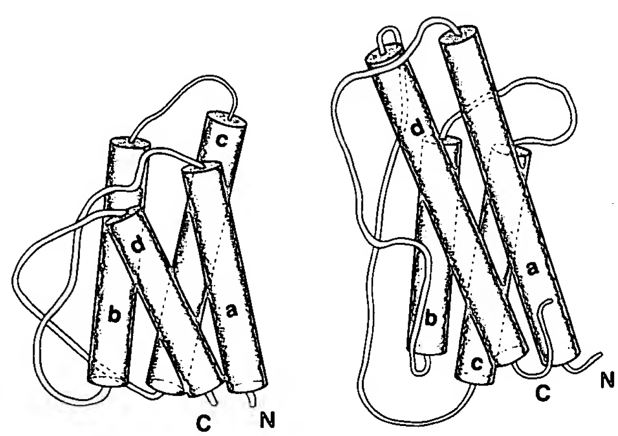

FIG. 1. Four α-helical bundle cytokines. Schematic drawing showing typical short-chain and long-chain four-helical bundle cytokines. Although these both exhibit an "up-up-down-down" topology to their four α helices, note that in the short-chain cytokines, the AB loop is behind the CD loop, whereas in the long-chain cytokines, the situation is reversed. Figure courtesy of Dr. Alex Wlodawer, National Cancer Institute.

short-chain cytokines include IL-2, IL-3, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-7, IL-9, IL-13, IL-15, monocyte-CSF (M-CSF), and stem cell factor (SCF), whereas the long-chain cytokines include growth hormone, prolactin, erythropoietin, thrombopoietin, leptin, IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), and G-CSF (Table 1) (8,9). In addition to a difference in the length of the helices, which typically are approximately 15 amino acids long for the short-chain helical cytokines and 25 amino acids long for the long-chain helical cytokines, there are differences in the angles between the pairs of helices, and the AB loop is "under" the CD loop in the short cytokines, but "over" the CD loop in the long cytokines (Fig. 1) (7,8,10). Short-chain, but not long-chain, cytokines have β sheet structures in the AB and CD loops. The groupings according to short-chain and long-chain cytokines have evolutionary considerations and also correlate with grouping of receptor chains for these two subfamilies of type I cytokines. An analysis of short-chain cytokines has shown that 61 residues comprise the family framework, including most of the 31 residues that contribute to the buried inner core. The similarities and differences in the structures of IL-2, IL-4, and GM-CSF have been carefully analyzed (6). Among these cytokines, there is considerable variation in the intrachain disulfide bonds that stabilize the structures. For example, IL-4 has three intrachain disulfide bonds, GM-CSF has two, and IL-2 has only one. In IL-4, the first disulfide bond (connecting residues 24 and 65) connects loop AB to BC, the second (connecting residues 46 and 99) connects helix B and loop CD, and the third (connecting residues 3 and 127) connects the residue preceding helix B with helix D. In GM-CSF, the N terminus of helix B and the N terminus of β strand CD are connected by one disulfide bond, whereas the other connects the C terminus of helix C and a strand following helix D. In IL-2, a single essential disulfide bond connects residues 58 and 105 to connect helix B to strand CD. Thus, each cytokine has evolved distinct disulfide bonds to stabilize its structure, although it is typical that helix B is connected to the loop between helices C and D. The structures formed by helices A and D are more rigorously conserved than those formed by helices B and C, primarily due to the interhelical angles; helix D and the connecting region are the most highly conserved elements among the three cytokines (6). This is of particular interest because the regions of type I cytokines that are most important for cytokine–cytokine receptor interactions (based on analogy to the growth hormone receptor structure) include helices A and D and residues in the AB and CD loops, whereas helices B and C do not form direct contacts (6).

Certain variations on these typical structures can occur. For example, IL-5 is unusual in that it is a dimer, positioned in such a fashion so that the ends containing the N and C termini are juxtaposed (11). Helix D is "exchanged" between the two covalently attached molecules so that helix D of each molecule actually forms part of the four-helix bundle of the other monomer (11). M-CSF is also a helical cytokine dimer, but no exchange of helix D occurs (10). The IFNs achieve related albeit somewhat different structures and also are known as type II cytokines (8). IFN-β has an extra helix that is positioned in place of the CD strand (12). IFN-γ is a dimer, each of which consists of six helices (13), as can be seen in Fig. 2. Two of these helices are interchanged, including one from each four-helix bundle (10,13). IL-10, which is closely related to IFN-γ, has a similar structure (14). It is interesting that the majority of helical cytokines have four exons, with helix A in exon 1,

TABLE 1. *Four helical-bundle cytokines*

Short-chain cytokines	Long-chain cytokines
IL-2	IL-6
IL-4	IL-11
IL-7	Oncostatin M
IL-9	Leukemia inhibitory factor
IL-13	CNTF
IL-15	Cardiotropin-1
IL-3	Growth hormone
IL-5[a]	Prolactin
GM-CSF	Erythropoietin (EPO)
	Thrombopoietin (TPO)
M-CSF[a,b]	Leptin
SCF[b]	G-CSF

[a]Dimers.
[b]Different from the other four helical bundle cytokines in the M-CSF and SCF receptors (CSF-1R and c-kit, respectively) have intrinsic tyrosine kinase activity and are not type I cytokine receptors.

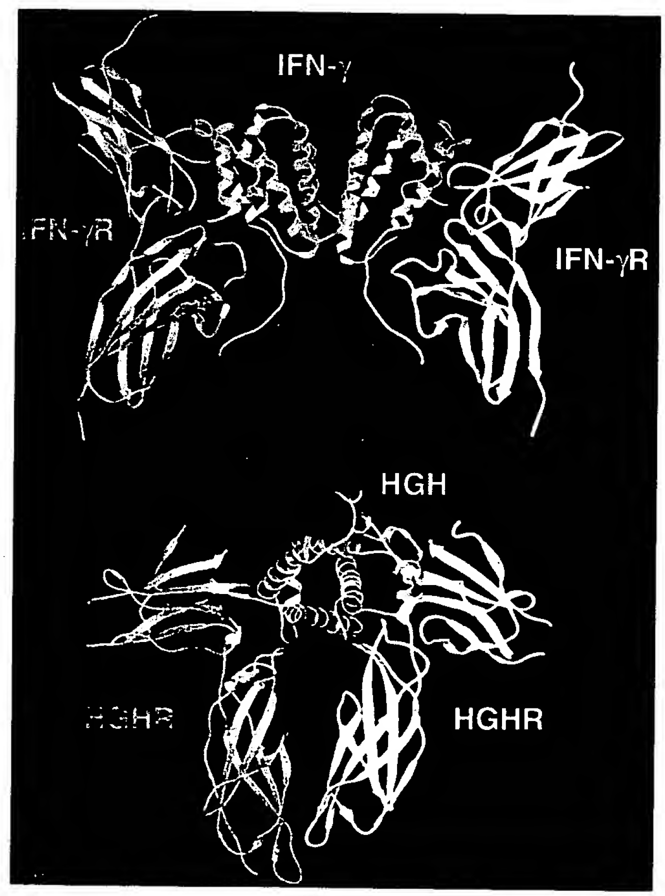

FIG. 2. Structure of the growth hormone and IFN-γ receptors. Shown are ribbon diagrams of the structures of the IFN-γ receptor (*above*) and growth hormone receptor (*below*) as examples of type II and type I cytokine receptors. In the IFN-γ receptor, only IFNGR-1 complexed to the IFN-γ dimer is shown because the full structure with IFNGR-2 is not available. For growth hormone, both growth hormone receptor monomers are shown. The coordinates for the growth hormone–growth hormone receptor structure are from ref. 21 and those for the IFN-γ–IFNGR-1 structure are from ref. 221. Figure courtesy of Dr. Alex Wlodawer, National Cancer Institute.

helices B and C in exon 3, and helix D in exon 4 (7). A related organization is found for IFN-γ, as well as the long-chain helical cytokines growth hormone and G-CSF. However, there are a number exceptions: for example, IL-15 is divided into five exons and IFN-β has only one exon, being devoid of introns (7).

Receptors for Type I Cytokines

The first published report suggesting that type I cytokine receptors had shared features came from a comparison of the sequences of the erythropoietin receptor and the IL-2 receptor β chain (15), but an analysis of a larger number of type I cytokine receptors provided a much clearer view of this new superfamily (16). Type I cytokine receptors are generally type I membrane–spanning glycoproteins (N-terminal extracellular, C-terminal intracellular), the only exceptions being proteins like the CNTF receptor α chain, which lacks a cytoplasmic domain and instead has a glycosylphosphoinositol (GPI) anchor; however, the orientation of this protein is otherwise similar to that of a type I membrane protein. In their extracellular domains, a number of conserved similarities have been noted (Table 2). These include four conserved cysteine residues that were predicted to be involved in intrachain disulfide bonding, and a tryptophan residue, located two amino acids C-terminal from the second conserved cysteine. In addition, a membrane proximal region WSXWS (trp-ser-X-trp-ser) motif was found to be generally conserved, although one exception to this relatively rigorous conservation is found in the growth hormone receptor, in which the motif is a substantially different YGEFS (tyr-gly-glu-phe-ser) sequence.

Interestingly, analysis of a number of the receptors showed that the two sets of cysteines are typically encoded in two adjacent exons, and the exon containing the WSXWS motif is typically just 5′ from the exon encoding the transmembrane domain. Although serines can be encoded by six different codons (i.e., six-fold degeneracy in codon usage), the codons used to encode the serines in WSXWS motif are far more limited, with two of the six possible codons (AGC and AGT) dominating. These data are consistent with a common ancestral precursor. Although many of the known cytokine receptors have been cloned based on expression cloning using a defined ligand, the limited degeneracy of the WSXWS motif has facilitated the complementary DNA (cDNA) cloning of new type I cytokine receptor members (via polymerase chain reaction [PCR]), leading to the first identification of IL-11R (17,18), IL-13Rα (19), and an oncostatin M receptor (20). Another shared feature of type I cytokine receptors is the presence of fibronectin type III domains. In some cases, such as the common cytokine receptor β chain (β_c), which is shared by the IL-3, IL-5, and GM-CSF receptors, the extracellular domain is extended, containing duplications of the domains comprising the four conserved cysteines and the WSXWS motif.

TABLE 2. *Features common to type I cytokine receptors*

Extracellular domain
Four conserved cysteine residues, involved in intrachain disulfide bonds
WSXWS motif
Fibronectin type III modules
Cytoplasmic domain
Box 1/Box 2 regions—the Box 1 region is a proline-rich region that is involved in the interaction of Janus family tyrosine kinases

Overall, the different receptor molecules, analogous to the cytokines, have extremely limited sequence identity. Nevertheless, they appear to form similar structures, based on the known structures for the growth hormone prolactin and erythropoietin receptors (21–23), as well as the modeling of other cytokine receptor molecules based on the known structures. Thus, the available data indicate closely related three-dimensional structures for the different type I cytokines and closely related structures for type I cytokine receptors, despite the widely divergent sequences. It is important to note, however, that the only type I cytokine receptors whose structures have been solved correspond to long-chain type I cytokines. The cytokines and their receptors have presumably coevolved, with the differences in amino acid sequences between different cytokines allowing for their distinctive interactions with their cognate receptor chains. At times, however, as illustrated below, despite amino acid differences, a number of sets of cytokines are capable of interacting with shared receptor chains, allowing a number of the different cytokines and their receptors to be grouped into subfamilies (8).

In addition to the above noted similarities in the extracellular domains, there are sequence similarities that are conserved in the cytoplasmic domain of cytokine receptors. In particular, a membrane-proximal region known as the Box 1/Box 2 region is conserved (Table 2), with a proline-rich Box 1 region being the most conserved (24). This will be discussed in greater detail below.

Type I Cytokine Receptors Are Homodimers, Heterodimers, or Higher Order Receptor Oligomers

The first cytokine receptor structure to be solved was that for growth hormone (Fig. 2) (21). Before the x-ray crystallographic analysis, it was believed that growth hormone bound to its receptor with a stoichiometry of 1:1. Remarkably, however, the x-ray crystal structure solution provided the first evidence that a single growth hormone molecule interacted with a dimer of the growth hormone receptor, in which each receptor monomer contributes a total of seven β strands. Perhaps the most striking finding was that totally different parts of the growth hormone molecule interacted with the same general region of each growth hormone receptor monomer. The three-dimensional x-ray crystal structure for growth hormone and its receptor is shown in Fig. 2. Solving the structure also clarified the basis for growth hormone receptor assembly (21). Kinetically, growth hormone is believed to interact first with one receptor monomer via a relatively large and high-affinity interaction surface (site I), spanning approximately 1,230 Å^2. A second receptor monomer then interacts with the growth hormone–growth hormone receptor complex via two contact points—one on growth hormone (spanning approximately 900 Å^2)(site II), and the other on the first receptor monomer (spanning approximately 500 Å^2) (site III), located much more proximal to the cell membrane. Thus, a total of three extracellular interactions are responsible for the formation of the growth hormone–growth hormone receptor complex. Logically, mutations in site I might prevent receptor binding, whereas mutations in site II would potentially prevent dimerization and signal transduction, providing a rational method for the design of antagonists.

The growth hormone receptor structure showed that the growth hormone receptor extracellular domain is composed of two